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ASSOCIATION BETWEEN INTERMALE SOCIAL AGGRESSION AND CELLULAR DENSITY WITHIN THE CENTRAL AMYGDALOID NUCLEUS IN RATS WITH LITHIUM/PILOCARPINE-INDUCED SEIZURES [1]

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Summary.—Aggressive behaviors (numbers of bites/hour) within groups (*ns* = 8) of normal rats and rats in which seizures had been induced by a single systemic injection of lithium/pilocarpine were observed for 11 successive, 1-hr. periods. Mean numbers of neurons and glial cells were counted for 10 different nuclei of the amygdala for 16 different brains (8 control; 8 seizure). Although there was no significant difference found between rats with chronically induced seizures and controls for the numbers of neurons per area within the central medial amygdaloid nucleus, the neuronal density was correlated significantly (.92) with mean numbers of bites per hour for the chronically epileptic group only. The hypothesis that seizure-induced damage within proximal amygdaloid nuclei disinhibits the central nucleus and encourages aggression was supported.

Extreme intermale aggression, as defined by body lacerations and significant tissue damage around the face and ears, begins to emerge in rats (Persinger, Bureau, Kostakos, Peredery, & Falter, 1993) within 10 to 15 days after seizures have been induced by a single systemic injection of lithium (3 mEq/kg) and pilocarpine (30 mg/kg). Although the distribution of the neuronal damage extends into extra-amygdaloid regions such as the thalamus (Bureau, Peredery, & Persinger, 1994), Bedard and Persinger (1995) have hypothesized that the disinhibition of the central nucleus of the amygdala is a major contributor to the enhanced aggression. This portion of the amygdala has been implicated as a significant modulator of intermale aggression in rodents for more than 30 years (Kaada, 1972).

Recently, Bedard and Persinger (1995) reported that the extreme aggression could be reduced and even eliminated by the consumption of the synthetic glucocorticoid prednisolone but not dexamethasome. Because the latter is sequestered primarily by only type 2 of the two subtypes of glucocorticoid receptors while the former is sequestered by both, aggression was attributed to increased sequestering of corticotrophin releasing hormone within the amygdaloid-hypothalamic pathways that contained this subtype of glucocorticoid receptor. The reduction of the aggression associated with oral pred-

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nisolone, which is also known to block corticotrophin-induced startle reflexes via the suprageniculate nucleus of the thalamus to the central amygdala (Liang, Melia, Campeau, Falls, Miserendino, & Davis, 1992), was attributed to its interaction with glucocorticoid type 1 and type 2 receptors within aggregates of neurons inside the perimeter of the central nucleus.

If portions of the central amygdaloid nucleus modulate the extreme aggression observed and this aggression is disinhibited by proximal neuronal necrosis subsequent to lithium/pilocarpine-induced seizures, then the numbers of neurons within this subset of amygdaloid neurons should be strongly correlated with quantitative measures of aggression. To test this hypothesis, the brains of eight normal and eight experimental Wistar male rats, 110 days of age (Charles River, Quebec) were evaluated. Seizure-induced brain damage had been produced in the latter group (90 days of age) by the subcutaneous injection of lithium chloride (3 mEq/kg) followed 4 hr. later by pilocarpine (30 mg/kg) and 30 min. later by 30 mg/kg of acepromazine. Eight of the rats received this procedure while the other eight received the Bureau method [seizure induction, acepromazine, and then 19 hr. of physical restraint, which facilitates hypothermia (Bureau & Persinger, 1993)]. Observations began about 21 days later. The protocol was approved by the local animal care committee.

The rats whose brains were studied had been maintained in groups of six (either all controls, all chronic epileptic rats, or an equal combination of these conditions) within one of two large plastic cages (Persinger, 1994). After 11 days of habituation, the experiment began. Each rat within each group had been observed for one hour per night during red-light conditions for 11 consecutive nights. The numbers of bites per hour for each rat had been counted. A biting (episode) was defined as dental contact with another rat that resulted in squeaking or tissue damage. These episodes were usually less than 1 to 2 sec. duration. At least 5 sec. of no biting were required before a subsequent behavior was scored as a second biting episode. The mean numbers of biting episodes per hour for the 11 days were calculated for each rat. There were three separate, successive blocks of observations for the (total of) six groups.

At the end of the experiment the brains were removed by decapitation, fixed in ethanol-formalin-acetic acid, and processed. The brains of the 16 rats (8 controls; 8 seizure–4 from the Bureau method) in this study were randomly selected from the total pool of rats (6×6 per cage $= 36$). Ten micrometer coronal sections, taken every 50 μm, were stained with toluidine blue O. The numbers of neurons (including those with eccentric nuclei or signs of chromatolysis) and glial cells (primarily astroglia) were counted per field at 1000 × magnification (oil immersion) for three to four fields for the following amygdaloid nuclei as defined by Paxinos and Watson (1986): me-

dial nucleus; medial nucleus, anterior part; medial nucleus, posterior ventral part; central nucleus, lateral part; central nucleus, medial part; basolateral nucleus, anterior part; basolateral nucleus, ventral part; basomedial nucleus; lateral nucleus; and lateral nucleus, ventral lateral part.

These particular structures were selected on the bases of their consistent occurrence within the sections and their involvement with aggressive behavior as defined by lesion studies. The intercorrelations between fields for the same structure ranged between .80 and .96. The mean numbers of neurons or glial (astroglia and oligodendroglia) per field for each structure was calculated and expressed as numbers of cells per mm-sq.

One-way analyses of variance were completed between the two groups for the numbers of neurons and glial cells per structure. Correlations between mean numbers of bites per hour and the mean numbers of neurons and glial cells were obtained. Glial/neuronal ratios were also calculated by dividing the numbers of glial cells per field by the numbers of neurons per field. All analyses employed SPSS software on a VAX 4000 computer.

The rats in which seizures had been induced exhibited significantly more ($F_{1,14}=7.00$, $p<.01$) bites per hour ($M=7.9$, $SD=5.4$) than did the controls ($M=2.7$, $SD=1.1$). The means and standard deviations for the numbers of neurons per mm-squared within the 10 structures of the amygdala for the rats in which seizures had been induced (about one month earlier) and the controls are shown in Table 1. The glial/neuron ratios for each of these structures for the two groups are shown in Table 2. Discriminative analyses

TABLE 1

NUMBERS OF NEURONS PER MM-SQ WITHIN DIFFERENT NUCLEAR GROUPS OF AMYGDALA FOR NORMAL RATS ($n=8$) AND RATS ($n=8$) IN WHICH SEIZURES HAD BEEN INDUCED BY LITHIUM/PILOCARPINE

Area	Control Group		Seizure Group		F
	M	SD	M	SD	
Medial Nucleus					
Principal	1731	308	1277	148	14.01*
Anterior	1090	117	973	124	3.77
Posterior Ventral	1549	296	1436	142	0.98
Central Nucleus					
Lateral	1361	162	1169	98	8.24*
Medial	1342	177	1325	114	0.04
Basolateral Nucleus					
Anterior	867	78	822	56	1.74
Ventral	721	122	701	115	0.10
Basomedial Lateral Nucleus	1380	376	1167	284	1.61
Principal	1196	260	1191	158	0.00
Ventrolateral	830	125	763	83	0.22

*$p<.01$.

(canonical correlation = 0.93; $\chi^2 = 27.43$, $p < .001$) indicated that only one measure, the glial/neuronal ratio within the principal medial nucleus resulted in 100% correct classification of the 16 rats.

TABLE 2
RATIOS OF NUMBERS OF GLIAL CELLS/NEURONAL CELLS (1000 ×) FOR VARIOUS AMYGDALOID REGIONS FOR CONTROL RATS ($n = 8$) AND RATS ($n = 8$) WITH SEIZURE-INDUCED BRAIN INJURY

Area	Control Group		Seizure Group		F
	M	*SD*	*M*	*SD*	
Medial Nucleus					
Principal	0.39	0.10	1.01	0.15	92.10†
Anterior	0.63	0.15	0.98	0.22	11.95*
Posterior Ventral	0.40	0.07	0.74	0.20	19.57†
Central Nucleus					
Lateral	0.50	0.07	0.77	0.08	48.27†
Medial	0.57	0.03	0.75	0.11	19.44†
Basolateral Nucleus					
Anterior	0.70	0.10	0.80	0.12	2.91
Ventral	0.90	0.16	1.28	0.21	16.75†
Basomedial Lateral Nucleus	0.53	0.14	1.08	0.15	53.10†
Principal	0.56	0.15	0.77	0.14	7.69
Ventrolateral	0.86	0.20	1.72	0.37	33.10†

*$p < .01$. †$p < .001$.

Fig. 1 shows the correlation between the mean numbers of bites per hour for the rats with the seizure history and the numbers of cells within the central nucleus, medial portion; the Spearman *rho* was .89 ($p < .001$). This association was not significant statistically ($r = .45$, $p > .05$) for the control rats. Partial correlation between the neuronal density within the central nucleus, medial portion and either the neuronal density or glial/neuronal ratios for each of the other structures did not reduce the correlation to values below 0.85.

The present results supported the hypothesis that disinhibited activity within the central amygdaloid nucleus strongly contributes to the extreme (tissue-damaging) intermale aggression that has been observed in rats within a few weeks after seizures were induced by a single subcutaneous injection of lithium and pilocarpine. That the numbers of neurons within the experimental rats' central amygdalas did not differ from those of the controls but were still positively and strongly associated with aggression indicates that the normal (preseizure) individual variation in the numbers of cells within this region determines the severity of the aggression. Stated alternatively, the intrinsic capacity for aggression became evident when the inhibitory input from other regions was damaged by the seizures.

Gliosis, defined as increased density of glial cells within a region of

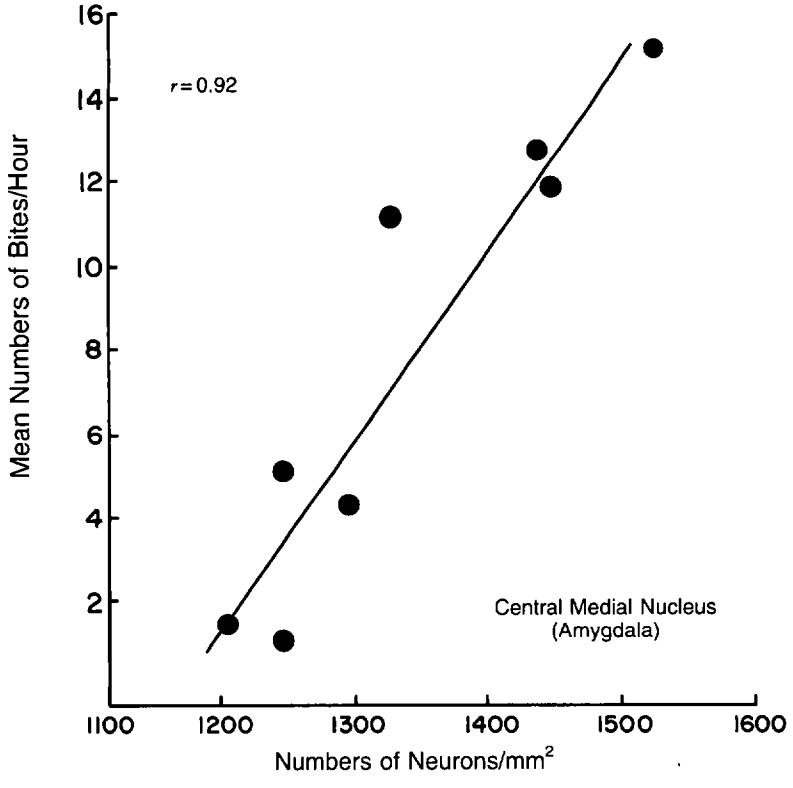

FIG. 1. Numbers of bites per hour and the numbers of neurons per square mm within the central nucleus, medial portion of the amygdala in rats in which seizures had been induced by a single subcutaneous injection of lithium and pilocarpine

anomalously reduced neuronal size or numbers (which we assume was responsible for the increased glial/neuronal ratios that were noted in nine of the ten structures, including the ratio that was strongly correlated with aggression), was more pervasive than we anticipated by lower magnitude (400 ×) light microscopy. The similarity in neuronal density between the control and the seizured rats for most of the amygdaloid nuclei together with the appearance of strong gliosis in the latter condition suggests that alterations had occurred within the organization of dendrites or axon terminals. Because axonal terminals from different neurotransmitter sources converge upon target dendrites and small groups of neurons within the larger aggregates (that define traditional structures), the altered behavior would be secondary to the alteration in the baseline ratios of input from these different sources. We think that the protracted seizures resulted in "autolesions" when the cholinergic pathways were overstimulated and destroyed by the

initial seizure. The qualitative shifts in microdistributions of the glial cells suggest that only specific types of terminals and receptor classes may have been affected.

We have not determined which subset of neurons within specific amygdaloid nuclei are vulnerable to the seizure-induced brain injury. Rats that are injected with slightly lower dosages of lithium and pilocarpine and do not display overt seizures do not exhibit any discernable brain injury (at least at 100 ×). The conspicuous reduction in neuronal density within the principal medial nucleus of rats in which limbic motor seizures were induced is revealing since this structure is the main gateway for thalamic afferents from the paraventricular nucleus (bilaterally), paratenial nucleus, ventral posteromedial nucleus, ventral posterolateral nucleus, posterior group and suprageniculate nucleus (de Olmos, Alheid, & Beltramino, 1986). These thalamic regions are extensively damaged (Persinger, Bureau, & Peredery, 1994) and would promote transneuronal degeneration. The expected correlated loss of afferent terminals would encourage gliosis. The glial/neuronal ratios within the principal medial nucleus were sufficient to classify correctly all (100%) of the experimental and control rats. If our interpretation of transneuronal degeneration is correct, then the mild but statistically significant decrease in neuronal density within the central nucleus, lateral part would be due to the massive damage within the paratenial nucleus whose fibers preferentially innervate this portion of the amygdala rather than the medial group.

Although we have not stained for neurotransmitters, the morphological data are compatible with our hypothesis that the corticotrophin releasing factor, an intrinsic epileptogenic compound, is involved with the observed aggression. Cell bodies containing the corticotrophin releasing factor are found within both the central amygdala and medial groups and these groups (de Olmos, *et al.*, 1986) send strong input to the hypothalamus, particularly the paraventricular nuclei. Whether this mechanism is involved with "stress-induced" irritability and aggression in human males is still to be determined.

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